and complete mixing of the liquids. Shake the flasks intermittently for 1 hour. Proceed as directed in paragraph (e) of this section.

(2) *Finished product solutions.* Prepare the sample for assay as directed in the individual section for each antibiotic product to be tested.

(e) *Procedure.* Inject 2.5 microliters of each solution into the gas chromatograph. Use the conditions and materials listed in paragraphs (a), (b), and (c) of this section. The conditions should be adequate to maintain a stable base line and provide at least 60 percent deflection of the recorder scale by the spectinomycin peak. The resolution of the peaks should be complete. The internal standard will be eluted before spectinomycin. Calculate the spectinomycin content as directed in paragraph (f) of this section.

(f) *Calculations.* Calculate the spectinomycin content of the sample as follows:

$$\text{Micrograms of spectinomycin per milligram} = \frac{R_u \times W_s \times f}{R_s \times W_u}$$

where:

R_u=Area of spectinomycin sample peak (at a retention time equal to that observed for the spectinomycin standard)/Area of internal standard peak;

R_s=Area of the spectinomycin standard peak/Area of internal standard peak;

W_s=Weight of the spectinomycin working standard in milligrams;

W_u=Weight of the sample in milligrams;

f=Potency of the spectinomycin working standard in micrograms per milligram.

§ 436.308 Paper chromatography identity test for tetracyclines.

(a) *Equipment*—(1) *Sheet* (*chromatographic*). Whatman No. 1 filter paper for chromatography, 20 × 20 centimeters.

(2) *Chamber* (*chromatographic*). Cylindrical glass chromatographic jar, 25 centimeters high by 12 centimeters in diameter, with a ground-glass lid.

(3) *Preparation of solutions*—(i) *pH3.5* buffer. Mix 13.93 volumes of 0.1*M* citric acid with 6.07 volumes of 0.2*M* of disodium phosphate.

(ii) *Solvent* (*organic phase*). Mix chloroform, nitromethane, and pyridine in volumetric proportions of 10:20:3, respectively.

(b) *Preparation of spotting solutions.* Prepare solutions of the working standard and sample as follows: Accurately weigh a portion of the working standard and sample and dilute with methanol to obtain a concentration of 1 milligram per milliliter of antibiotic to be tested.

(c) *Procedure.* Fill the chamber to a depth of 0.6 centimeter with freshly prepared solvent. Draw a starting line about 2.5 centimeters from and parallel to the bottom of the sheet. Wet the sheet thoroughly with the pH 3.5 buffer and blot it firmly between sheets of absorbent paper. Starting about 5 centimeters from the edge of the sheet and at 1.5-centimeter intervals, apply to the starting line 2 microliters each of standard solution, sample solution, and a 1:1 mixture of the standard and sample solutions. Allow a few minutes for the sheet to dry partially, and while still damp place it in the chamber with the bottom edge touching the solvent. When the solvent front has risen about 10 centimeters, remove the sheet from the chamber. Expose the paper to ammonia vapor. Examine the dried sheet under a strong source of ultraviolet light and record the position of any fluorescent spots. Measure the distance the solvent front traveled from the starting line and the distance that the fluorescent spots are from the starting line. Calculate the R_f value by dividing the latter by the former.

[39 FR 18944, May 20, 1974, as amended at 44 FR 30333, May 5, 1979; 45 FR 16472, 16474, Mar. 14, 1980]

§ 436.309 Anhydrotetracyclines and 4-epianhydrotetracycline.

Determination of 4-epianhydrotetracycline and anhydrotetracyclines in tetracycline, tetracycline hydrochloride, tetracycline phosphate, and in dosage forms thereof is as follows:

(a) *Screening procedure for total anhydrotetracyclines content*—(1) *Sample solution preparation*—(i) *Bulk packaged for repacking or for use in the manufacture of another drug.* Accurately weigh approximately 50 milligrams of the sample into a 50-milliliter volumetric flask and add 10 milliliters of 0.1*N* hydrochloric acid. Shake until sample is